AD_____

Award Number: W81XWH-10-1-0410

TITLE: PPAR delta as a therapeutic target in triple-negative breast cancers

PRINCIPAL INVESTIGATOR: Chao Qi, Ph.D.

CONTRACTING ORGANIZATION: Northwestern University
Evanston, IL 60208

REPORT DATE: September 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2011	Annual	1 September 2010 – 31 August 2011

4. TITLE AND SUBTITLE

PPAR delta as a therapeutic target in triple-negative breast cancers

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-0410

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Chao Qi

E-Mail: cqi451@northwestern.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Northwestern University
Evanston, IL 60208

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

PPARδ is a member of peroxisome proliferators activated receptors which play important role in regulating cellular metabolisms. Basal-like breast cancers accounting for ~15% of all breast cancer cases tend to be very aggressive and have poor prognosis. Basal-like breast cancers are considered to be derived from mammary stem cells. Abnormal activation of Wnt signaling is often found in human basal-like breast cancers. Here we report that PPARδ expression is induced by Wnt signaling in mammary stem cells. Activation of PPARδ by its ligands and fatty acids promotes the proliferation of mammary stem cells. Activation of PPARδ induces the proliferation of mammary cancer stem cells. PPARδ agonist accelerates the onset of tumors and increases the growth rate of tumors in MMTV-Wnt1 transgenic. We also found that PPARδ antagonist inhibits the proliferation and anchorage-independent growth of a human basal-like breast cancer cell line.

15. SUBJECT TERMS
Breast carcinoma; triple negative breast cancer; cancer stem cells; PPARD.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	12	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

Introduction……..1

Body……..2

Key Research Accomplishments…… . ….. 5

Reportable Outcomes…… 6

Conclusion……7

References……8

Appendices……9

INTRODUCTION

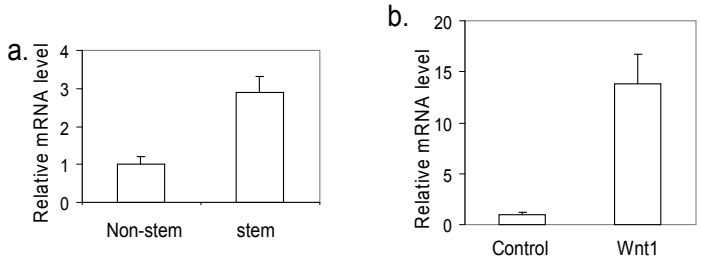
PPARδ is a member of peroxisome proliferators activated receptors which play important role in regulating cellular metabolisms. PPARδ can be activated by fatty acids, triglycerides, and prostacyclin. PPARδ has been implicated in the colorectal carcinogenesis by numerous observations. PPARδ agonist was shown to promote chemical carcinogen-induced mammary tumorigenesis. Basal-like breast cancers accounting for ~15% of all breast cancer cases tend to be very aggressive and have poor prognosis without effective treatment. Basal-like breast cancers are considered to be derived from mammary stem cells. Wnt signaling is required for the self-renewal of mammary stem cells. Overexpression of Wnt1 in mice induces the development of mammary tumors which most likely originate from stem/progenitor cells as the tumors contain both luminal and myoepithelial cells. Abnormal activation of Wnt signaling is often found in human basal-like breast cancers. The objectives of this proposal are to define the function of PPARδ in mammary stem cells and basal-like breast cancer development.

BODY

Task 1. Determine the function of PPARD in the proliferation of cancer stem cells from triple-negative breast cancer mouse model.

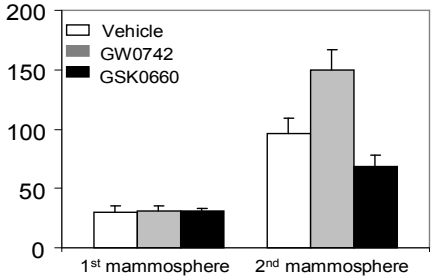
Wnt signaling induces PPARδ expression in mammary stem cells. The mammary COMMA-1D cell line contains mammary stem cells. Our microarray analysis revealed that PPARδ expression was dramatically increased when COMMA-1D cells expressed the activated form of β-catenin. Wnt signaling has been shown to be active in mammary stem cells. Therefore, we examined PPARδ expression in mammary stem cells. PPARδ expression was increased in mammary stem cell population ($CD24^{med}$ and $CD49f^{high}$) compared with other non-mammary stem cells (Fig. 1a). When $CD24^{med}$ and $CD49f^{high}$ mammary epithelial cells from wild type mice were plated with irradiated NIH3T3 cells expressing Wnt1 or control cells as feeder cells, real time RT-PCR revealed that PPARδ mRNA was markedly induced by Wnt1 (Fig. 1b). As the level of PPARδ mRNA in NIH3T3 cells expressing Wnt1 was similar to that in control NIH3T3 cells (data not shown), this result indicated that PPARδ expression is induced in mammary stem cells by Wnt signaling.

Fig.1. (a), Increased expression of PPARδ in mammary stem cell population compared with non-stem cells, as revealed by real-time RT-PCR. Mammary stem cell population ($CD24^{med}$ and $CD49f^{high}$) from wild type mice were sorted out by flow cytometry. (b), Induction of PPARδ expression by Wnt1. Real time RT-PCR was performed with RNA prepared from mammary stem cells co-cultured with NIH3T3 expressing Wnt1 or control cells.



Activation of PPARδ induces the proliferation of mammary stem cells. When cultured in suspension, individual mammary stem cells form mammospheres, which are composed of myoepithelial cells, luminal epithelial cells, and stem cells. We found that the number and size of primary mammospheres were not affected by the treatment of PPARδ agonist GW0742 or antagonist GSK0660. However, the number of secondary mammospheres was increased with the treatment of PPARδ agonist and decreased by PPARδ antagonist (Fig. 2), suggesting that activation of PPARδ induces the proliferation of mammary stem cells.

Fig. 2. PPARδ agonist increased while antagonist decreased the proliferation of mammary stem cells. Mammary epithelial cells were subjected to suspension culture for mammosphere formation and treated with chemicals as indicated. The primary mammospheres were trypsinized, and equal number of cells was cultured for secondary mammosphere formation.



Arachidonic acid is more potent in inducing mammary stem cell proliferation than GW0742. As fatty acids act as agonists for PPARδ, we examined if fatty acids could also promote proliferation of mammary stem cells. When mammary epithelial cells were treated with arachidonic acid, even more secondary mammospheres were produced compared with those treated with GW0742 (Fig. 3a). Transient transfection assay revealed that while antagonist GSK0660 completely blocks the transactivation of PPARδ by GW0742, it only partially blocks PPARδ activity induced by arachidonic acid (Fig. 3b). PPARδ carries activation domain 1 (AF1) which unlike AF2 can not be inhibited by an

antagonist. Therefore, we tested the effect of arachidonic acid on the activity of AF1. Arachidonic acid was found to potentiate the AF1 transcriptional activity (Fig. 3c).

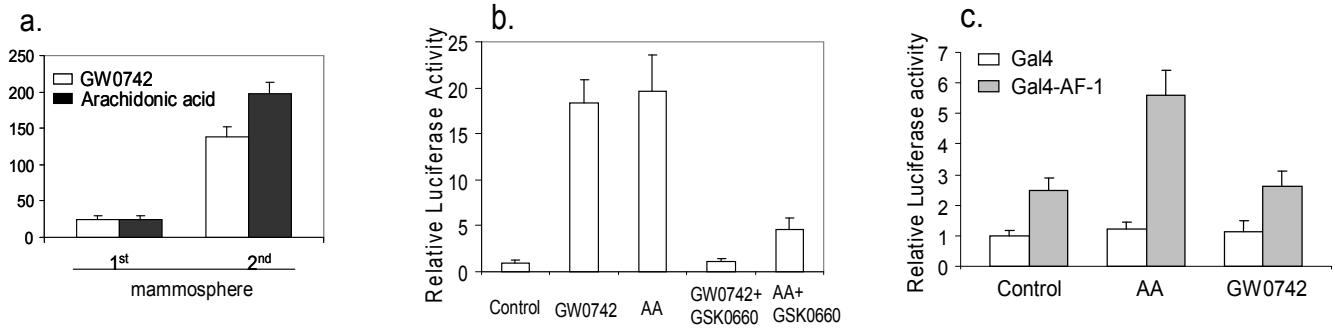
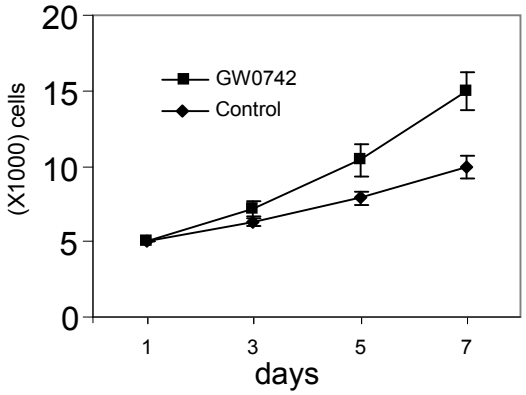


Fig. 3. (a). Arachidonic acid is more potent in inducing the proliferation of mammary stem cells than GW0472. Mammary epithelial cells were cultured for primary and secondary mammosphere formation and treated with chemicals as indicated. (b). Activation of PPARδ by arachidonic acid (AA) can only partially be b locked by its antagonist. PPARδ expression vector and PPRE-Luc reporter was cotransfacted into CV-1cells and treated with chemicals as indicated. (c). Arachidonic acid (AA) potentiates AF-1 activity of PPARδ. Vectors expressing Gal4 DNA binding domain or fusion protein between Gal4 DNA binding domain and AF-1 of PPARδ along with UAS-Luc reporter was contransfected into CV-1 cells and treated as indicated.

Activation of PPARδ induces the proliferation of mammary cancer stem cells. Mammary tumors were isolated from MMTV-Wnt1 transgenic mice. The tumors were trypsinized to obtain single cells. and labeled with anti-CD24/CD49f. Cancer stem cell popul ation ($CD24^+$ $CD49f^+$) were sorted out by flow cytometry. Eq ual number of c ancer stem cells was treated with control vehicle or agonist GW0742. Viable cells were counted once per two days for six days. The cells showed increased proliferation with the treatment of GW0742 (Fig. 4).

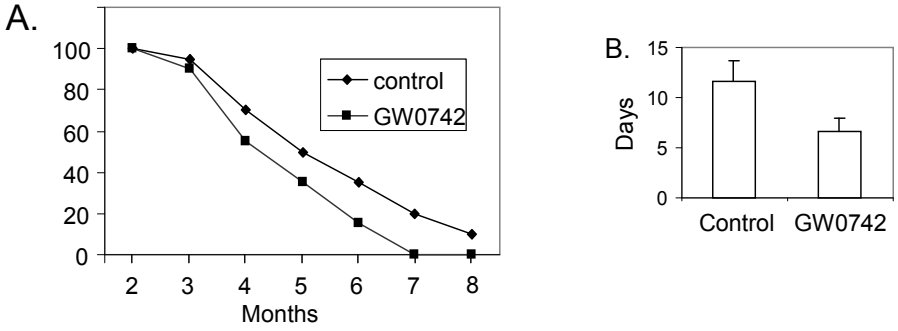
Fig. 4. PPARδ agonist increased the proliferation of cancer stem cells. Equal number of cancer stem cells isolated through flow cytometry sorting was cultured with the treatment of GW0742 or control vehicle and counted for six days.



Task 2. Evaluate the function of PPARD in triple-negative breast cancer mouse model.

Activation of PPARδ promotes the tumorigenesis of MMTV-Wnt1 transgenic mice. MMTV-Wnt1 transgenic mouse is a model for human triple-negative breast cancers. We treated MMTV-Wnt1 mice with PPARδ agonist GW0742, or control vehicle (20 mice for e ach group). GW0742 promoted the development of mammary tumors (Fig. 5). The histology of the tumors from GW0742 -treated mice and control mice was similar (data not shown). Therefore, activation of PPARδ promotes the tumorigenesis of MMTV-Wnt1 transgenic mice. Because of the strong oncogenic effect of Wnt1, the effects of PPARδ ligands on tumor development are relatively modest. However, for human breast cancers taking decades to develop, the impact of PPARδ activation by fatty acids from high fat diets could be far stronger.

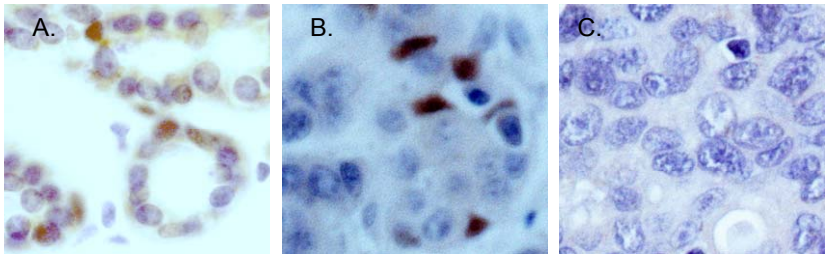
Fig. 5. PPARδ agonist promotes the development of mammary tumors. (A). The onset of tu mors in mice treated with GW0742 compared with control mice. (B). The growth rate of tumors treated with GW0742, as revealed by the time taken by tumors to reach a size of 1 cm. Differences in tumor onset and growth were statistically significant (P<0.05).



Task 3. Determine the function of PPARD in the proliferation of cancer stem cells from human triple-negative breast cancer cell lines.

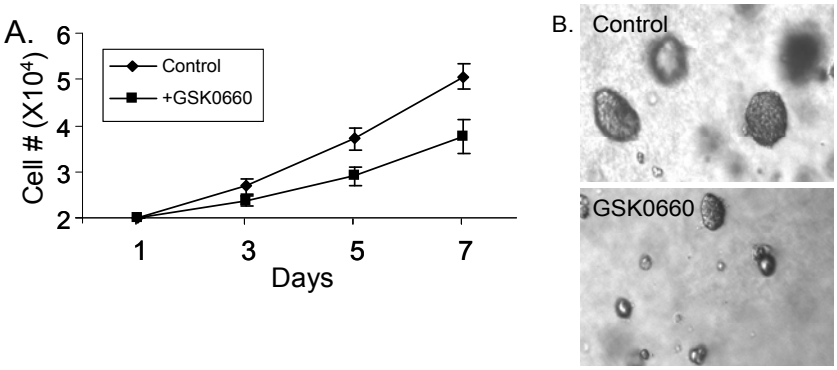
Strong expression of PPARδ in few tumor cells in some basal-like breast cancers. Immunohistochemistry revealed that PPARδ was expressed in both cytoplasm and nucleus in normal breast epithelial cells with strong nuclear expression in some basal cells (Fig. 6A). We examined the expression of PPARδ in human basal-like breast cancers. High level of PPARδ protein was detected in the nuclei of only few tumor cells in 4 of 10 tumors examined (Fig. 6B for representative picture). The few tumor cells with strong PPARδ expression could represent cancer stem cells.

Fig. 6. Immunostaining with anti-PPARδ. (A), normal breast tissue; (B), basal-like breast cancer expressing PPARδ; (C), breast cancer not expressing PPARδ.



Suppressed proliferation of basal-like breast cancer by PPARδ antagonist. The HCC1937 is basal-like breast cancer cell line . Treatment of HCC1937 cells with PPARδ antagonist GSK0660 suppressed their proliferation (Fig. 7A). Fu rthermore, the anchorage-independent growth of HCC1937 cells was strongly inhibited by GSK0660 (Fig. 7B). Similar results were obtained from another cell line BT20.

Fig. 7. (A). PPARδ antagonist inhibits the proliferation of HCC1937 cells. HCC1937 cells were plated at 2x10 ^{4} cells per well and treated with 1μM GSK0660 or control vehicle. V iable cells were counted by trypan blue staining. (B). PP ARδ antagonist suppresses the anch orage-independent growth of HCC1937 cells. HCC1937 cells were seeded in soft agar and treated with 1μM GSK0660 or control vehicle.



KEY RESEARCH ACCOMPLISHMENTS

* We report that PPARδ expression is induced by Wnt signaling in mammary stem/bi-progenitor cells. Activation of PP ARδ by its ligands and fatty acids promotes the proliferation of mammary stem/bi-progenitor cells and fatty acids are more potent by potentiating both the N-terminal activation function AF1 and the ligand-induced activation function AF2 of PPARδ. Activation of PPARδ induces the proliferation of mammary cancer stem cells.

* PPARδ agonist accelerates the onset of tumors and increases the growth rate of tumors in MMTV-Wnt1 transgenic.

* We also found that PPARδ antagonist inhibits the proliferation and anchorage-independent growth of a human basal-like breast cancer cell line.

REPORTABLE OUTCOMES
None

CONCLUSIONS

We report that PPARδ expression is in duced by Wnt signaling in mammary stem/bi-progenitor cells. Activation of PP ARδ by its ligands and fatty acids promotes the proliferation of mammary stem/bi-progenitor cells and fatty acids are more potent by potentiating both the N-terminal activation function AF1 and the ligand-induced activation function AF2 of PPARδ. Activation of PPARδ induces the proliferation of mammary cancer stem cells. PPARδ agonist accelerates the onset of tumors and increases the growth rate of tumors in MMTV-Wnt1 transgenic. We also found that PPARδ antagonist inhibits the proliferation and anchorage-independent growth of a human basal-like breast cancer cell line.

REFERENCES

None

APPENDICES

None.